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Patty Wilson

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Continued Prosecution Application

Under 37 C.F.R. § 1.53(d) Based On:

Application of: LeCluyse, Edward L., et al. Group Art Unit: 1651

Serial No.: 09/527,352 Examiner: Afremova, V.

Filed: March 17, 2000 Docket No.: 421/17/2

For: METHOD OF SCREENING CANDIDATE COMPOUNDS FOR SUSCEPTIBILITY TO BILIARY EXCRETION

* * * * * * * * * * * * *

DECLARATION PURSUANT TO 37 C.F.R. §§1.131-1.132

Commissioner of Patents
Washington, D.C. 20231

Sir:

1. I, Kim L.R. Brouwer, am a co-inventor of the invention disclosed and claimed in the subject above captioned U.S. Patent Application Serial No. 09/527,352.

2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit A**.

3. I have had an opportunity to review pending claims 67-200 in the above-referenced U.S. patent application.

4. I have also reviewed the following document: Liu et al., "Biliary Excretion in Sandwich-Cultured (SC) Hepatocytes: A Novel *In Vitro* Model System for Investigating Biliary Excretion," *Pharm. Sci.* 1:S-119 (1998) (Abstract – herein after referred to as Liu et al. [CC]).

5. The invention embodied in claims 105-118 of the subject U.S. patent application was invented prior to the November 16, 1998 publication date of Liu et al. [CC].

6. Attached hereto as **Exhibit B** is a true and accurate copy of a thesis draft prepared by my co-inventor Xingrong Liu while at the University of North Carolina at Chapel Hill. Dr. Liu's dissertation was defended on June 29, 1998. The draft dissertation was provided to the graduate committee at the University of North Carolina at Chapel Hill two weeks prior to this defense date. **Exhibit B** describes the invention embodied in claims 105-118 and predates the November 16, 1998 publication date of Liu et al. [CC]. Please note that the biliary clearance value was defined on page 149, and was discussed throughout Chapter 5, with the greatest detail provided on page 157.

7. I have also reviewed the following documents: LeCluyse et al. (1994) *Am. J. Physiol.* 266:C1764-C1774; Liu et al. (1997) *Pharm. Res.* 24:S-459; U.S. Patent No. 5,602,026 to Dunn et al.; Liu et al. (1996) *Pharm. Res. Init.* 13:S-393 (8003); and Poole et al. (1990) *Archives of Toxicology* 64:474-481 (hereinafter referred to respectively as: LeCluyse et al. [U]; Liu et al. [EE]; Liu et al. [DD]; Poole et al. [V]; and Dunn et al. [A]).

8. The LeCluyse et al. [U] journal article does not teach the quantitation of the excretion of a compound. The LeCluyse et al. [U] is merely concerned with showing a method of culturing hepatocytes to form canalicular networks so as to attempt to provide a representative model to study hepatic morphology and physiology.

9. Dunn et al. [A] does not teach any evaluation of biliary excretion. Liu et al. [EE], Poole et al. [V], and Liu et al. [DD] measure biliary excretion in terms of a biliary excretion index, a percentage of radiolabeled hormone accumulation, and K_m and V_{max}, respectively.

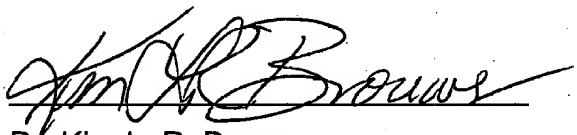
10. LeCluyse et al. [U], Liu et al. [EE], Poole et al. [V], and Liu et al. [DD] do not determine a biliary clearance value, and the biliary clearance value recited in claim 105 is clearly distinct from the approaches described in each of these documents. Biliary clearance is a function of intrinsic biliary clearance and the hepatic plasma flow rate. Compound cleared from blood or plasma into bile involves two processes: uptake across the sinusoidal membrane into the hepatocyte, and excretion across the canalicular membrane into bile. Biliary clearance represents the

volume of blood or plasma completely cleared of substrate that is excreted into bile per unit time, and will be determined by the rate-limiting step in the sequential processes (either uptake or excretion). In contrast, the biliary excretion index determines the fraction of accumulated substrate that appears in bile; thus this calculation only considers transport across the canalicular membrane. To further demonstrate the distinct nature of these calculations, consider the situation where substrate uptake is altered. In this case, if uptake is rate-limiting, the biliary clearance would be altered, but no change in the biliary excretion index would be observed.

11. A true and accurate plot showing a linear correlation of the *in vitro* biliary clearance value to *in vivo* biliary clearance is attached as **Exhibit C**. This plot was prepared using an experimental approach corresponding to that employed to prepare Figure 6B of the subject U.S. Patent Application Serial No. 09/527,352 as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,



Dr. Kim L. R. Brouwer

May 22, 2003

Date

Enclosures: Exhibits A-C

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Brouwer, Kim L. Rowse	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Oregon State University, Corvallis, OR	B.S.	1978	Pharmacy
University of Kentucky, Lexington, KY College of Pharmacy	Pharm.D. Ph.D.	1981 1983	Clinical Pharmacy Pharmacokinetics/ Pharmaceutical Science
University Kentucky, Lexington, KY College of Medicine, Dept. of Pharmacology	Post-Doctoral	1986	Drug Metabolism/ Pharmacology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

PROFESSIONAL EXPERIENCE:

1978-1981 Hospital Pharmacy Residency; A.B. Chandler Medical Center, University of Kentucky
1978-1983 Graduate Teaching/Research Assistant; College of Pharmacy, University of Kentucky
1983-1986 Postdoctoral Fellow, College of Medicine, Department of Pharmacology, University of Kentucky
1997-present Professor, Div. of Drug Delivery & Disposition, School of Pharmacy, University of North Carolina (Assistant, 1986-1992; Associate, 1992-1997)
1997-present Professor, Curriculum in Toxicology, School of Medicine, University of North Carolina (Assistant, 1987-1992; Associate, 1992-1997)
1996-present Director of Graduate Studies, School of Pharmacy, University of North Carolina at Chapel Hill

HONORS: National Rho Chi Graduate Scholarship, 1978; National Alpha Lambda Delta Alice Crocker Lloyd Graduate Fellow, 1980; American Foundation for Pharmaceutical Education Fellow, 1981-1982; Smith Kline Beckman Pharmaceutics/Biopharmaceutics AFPE Fellow, 1982-1983; American Association of Colleges of Pharmacy Young Investigators Program Grant Recipient, 1986-1987; PMA Foundation Research Starter Grant Recipient, 1987; Hollingsworth Faculty Scholar, UNC School of Pharmacy, 1995-1997; Fellow, American Association of Pharmaceutical Scientists, 1998; NIH Pharmacology Study Section Member, 1998-2002; PhRMA Foundation Award in Excellence in Pharmaceutics, 2001.

PUBLICATIONS: (selected from 87 refereed articles, 128 published abstracts, 3 book chapters)

Brouwer, KLR and Jones, JA: Altered Hepatobiliary Disposition of Acetaminophen Metabolites Following Phenobarbital Pretreatment and Renal Ligation: Evidence for Impaired Biliary Excretion and a Diffusional Barrier. *J Pharmacol Exp Ther* 252:657-664, 1990.

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Letrent, SP, Pollack, GM, Brouwer KR and Brouwer, KLR: Effect of GW918, a Potent P-Glycoprotein Inhibitor, on Morphine Pharmacokinetics and Pharmacodynamics in the Rat. *Pharm Res* 15:599-605, 1998.

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Xiong, H, Turner, KC, Ward, ES, Jansen, PLM and Brouwer, KLR: Altered Hepatobiliary Disposition of Acetaminophen Glucuronide in Isolated Perfused Livers from Multidrug Resistance-Associated Protein 2-Deficient TR- Rats. *J Pharmacol Exp Ther* 295:512-518, 2000.

Ward, ES, Pollack, GM and Brouwer, KLR: Probenecid-Associated Alterations in Valproic Acid Pharmacokinetics in Rats: Can *In Vivo* Disposition of Valproate Glucuronide Be Predicted from *In Vitro* Data? *Drug Metab Dispos.* 28:1433-1439, 2000.

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Zamek-Gliszczynski, MJ, Xiong, H, Patel, NJ, Turncliff, RZ, Pollack, GM, and Brouwer, KLR: Pharmacokinetics of 5 (and 6)-Carboxy-2',7'-Dichlorofluorescein and Its Diacetate Promoiety in the Liver. *J Pharmacol Exp Ther* 304: 801-809, 2003.

McRae, MP, Brouwer, KLR, and Kashuba, AK: Cytokine Regulation of P-Glycoprotein. *Drug Metab Rev* in press, 2003.

Patel, NJ, Zamek-Gliszczynski, MJ, Zhang, P, Han, Y-H, Jansen, PLM, Meier, PJ, Stieger, B, and Brouwer, KLR: Phenobarbital Alters Hepatic Mrp2 Function by Direct and Indirect Interactions. *Mol Pharm* in press, 2003.

RESEARCH PROJECTS ONGOING OR COMPLETED DURING THE LAST THREE YEARS:

Ongoing Research Support

Title: Altered Hepatic Disposition of Anionic Drugs: Mechanisms
Principal Investigator: Brouwer, K.L.R.
Agency: NIGMS, NIH.
Type: Research Grant (R01 GM41935-10-13) Period: 07/01/01-06/30/05

The objective of this research program is to develop a mechanistic understanding of how perturbations in hepatic transport systems influence overall hepatobiliary disposition of anionic drugs and derived metabolites. A multiexperimental approach utilizing *in vivo*, isolated perfused rat liver, and *in vitro* cellular systems, including both rat and human hepatocytes, is being used to elucidate mechanisms of altered function of hepatic organic anion transport systems, predict alterations in hepatobiliary drug disposition and drug transport interactions.

Title: P-Glycoprotein Induction: Kinetic/Dynamic Implications
Principal Investigator: Pollack, G.M.
Co-Investigator: Brouwer, K.L.R.
Agency: NIGMS, NIH.
Type: Research Grant (R01 GM61191 1-4) Period: 4/1/01-3/31/05

The long-term goals of this research program are to explore the hypothesis that inducers of P-glycoprotein cause clinically relevant alterations in the disposition and action of P-glycoprotein substrates.

Title: Development of an *In Vitro*, Moderate-Throughput Screening Assay to Predict Hepatobiliary Disposition of Drug Candidates
Principal Investigator: Brouwer, K.L.R.
Agency: Pfizer, Inc.
Type: Research Grant Period: 10/22/01-10/21/04

The objective of the studies outlined in this research project is to develop an *in vitro*, moderate-thoughput screening model for rat and human hepatocytes that could be used to: (1) identify compounds that undergo extensive hepatic uptake and biliary excretion, and (2) accurately predict *in vivo* biliary clearance of drugs in rats and humans.

Completed Research Support

Title: *In Vitro* Methods to Examine Hepatobiliary Disposition of BILN 2061 ZW
Principal Investigator: Brouwer, K.L.R.
Agency: Boehringer Ingelheim
Type: Research Grant Period: 02/01/02-01/31/03

The objectives of this research collaboration include: (1) development and application of *in vitro* methodology to predict the hepatobiliary disposition of selected compounds (BILN 2061 ZW and analogs), (2) evaluation of the correlation between the biliary clearance of selected compounds *in vitro* and *in vivo*, and (3) identification of the basolateral and canalicular hepatic transport proteins responsible for the hepatic uptake and biliary excretion of selected compounds. In addition, the function of hepatic transport proteins in human hepatocytes from diseased livers will be examined, pending availability of tissue.

Title: Testing Two Pfizer Compounds in TR⁻ Rats
Principal Investigator: Brouwer, K.L.R.
Agency: Pfizer, Inc.
Type: Research Contract Period: 02/28/00-05/27/00

The *in vivo* disposition and biliary excretion of two new chemical entities were characterized in control and mutant TR⁻ rats to determine if these compounds were substrates for Mrp2.